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Production of enriched lamb in biodegradable packaging

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Abstract:
The paper describes an environmentally safe technology for biofortifying lamb with target components in required concentrations packed in biodegradable film. To address the problem of micronutrient deficiency, we developed a biologically safe method of enriching lamb with organic iodine and selenium supplements. Introducing selenium and iodine feed supplements to the animals' diet increased the average daily growth of their live mass in experimental groups by 3.43, 6.72, and 14.92% in groups I (iodine), II (selenium), and III (iodine and selenium), respectively, compared to the control group (feed only). The analysis of immune-physiological status showed an increase in phagocytic number in experimental groups: by 5.1% ($P > 0.95$), 9.4%, and 14.5% in groups I, II, III, respectively. In addition, the highest phagocytic activity and phagocytic intensity were observed in animals of group II and group III, indicating their higher resistance to adverse environmental factors, compared to the control. There was an increase in iodine, selenium and zinc content in the lamb meat from the experimental groups grown on enriched diets. We recorded a higher concentration of the micronutrients in the lambs receiving "Yoddar-Zn" and "DAFS-25" supplements together (Zn – 980 µg/100 g; Se – 53.9 µg/100 g; I – 77.6 µg/100 g). We found that the contents of zinc, selenium, and iodine in 100 g of *m. Longissimus dorsi* were 8.2%, 77.0%, and 51.7%, respectively, of the required content in the daily diet. Thus, we can use this raw material to produce functional meat products. Packaging lamb in sodium alginate-based biodegradable film helped reduce moisture loss during storage.

Keywords: Lamb, environmentally-friendly technology, feed supplements, essential trace elements, iodine deficiency, biodegradable film, polysaccharides, sodium alginate

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INTRODUCTION

The project "Fundamentals of the Russian state policy for healthy nutrition of the population until 2020" aims to create a set of measures to meet health needs of different groups of the population in accordance with medical requirements and people's traditions, habits, and economic status [1]. Fortification, or the process of *in vivo* optimization of raw materials and later a final product, is a significant tool for functional and specialized nutrition, especially in the light of diet personification trend [2].

According to V.A. Tutelyan, academician of the Russian Academy of Sciences, human health is largely determined by nature, level, and structure of nutrition, which is reported to have a number of serious disorders. Malnutrition is the main factor that causes irreparable damage to health, several times more severe than environmental pollution. It is the cause why 70% of the Russian population is reported to lack vitamin C, 40% have â-carotene and vitamin A deficiency, nearly a third of the population are vitamin B deficient, and absolutely everyone is iodine and selenium deficient.

The shortage of essential substances in nutrition is one of the most important issues in Russia. Many regions lack vital trace elements, such as selenium and iodine, in soil and water and consequently in livestock products [5].

According to WHO, sheep farming, a supplier of raw material for the meat processing industry, is now the third largest in the world. The use of lamb for the production of functional products is highly promising due to its contents of biologically active substances, such as complete animal protein, bioactive peptides, minerals (zinc, iron, selenium), vitamins, and fatty acids [2].

One of the ways to obtain high quality lamb meat that can provide people with essential trace elements is *in vivo* optimization of the meat chemical composition by adding essential nutrients into lamb diets [6]. The main advantage of *in vivo* lamb enrichment is the elimination of negative effect (overdose), since the supplement has already been "approved" by animals [7].

Meat processing companies today are extremely interested in innovative technologies that increase production profitability. It could open the floodgates to the global market, which is timely in view of the sanctions imposed on Russia.

In this regard, introducing organic trace elements into protein-carbohydrate complexes for agricultural animals' diets is one of the safest and inexpensive methods to obtain enriched meat and dairy raw materials [7, 8]. In humans and animals, iodine is found in inorganic compounds (iodides) and organic (about 75% of total iodine) covalently related forms (thyroglobulin), iodized amino acids (monoiodthyrosine and diiodothyrosine), and iodine-containing hormones (thyroxine and triiodothyronine). Inorganic iodine bound by chemical covalent bond (by amino acid residues – tyrosine, histidine) is easily organized and absorbed by internal secretion organs (thyroid system). Iodotyrosines are synthesized in thyroid follicles as part of thyroglobulin. "Iodine organification" occurs in the thyroid gland, where the enzymatic binding of inorganic iodine to amino acids of the protein – thyroglobulin (iodization) – occurs every second. As a result of aromatic electrophilic substitution, iodide (J^-) is embedded into the molecule of aromatic amino acid (tyrosine), forming a strong covalent bond with carbon (C–J). In addition to the thyroid gland, "iodine organification" is also carried out in the mammary and salivary glands, as well as other tissues and organs, though to a smaller degree [9].

Due to some circumstances, about 12% of total lamb production in Russia is industrially processed. The rest is sold mainly in unpacked carcasses, which results in mass loss and quality degradation [8].

Polyethylene, Saran (polyvinylidene chloride), and viscose polymer film materials protect the product from external influences, which improves the sanitary condition of meat and reduces its mass loss and bacterial seediness, promotes color preservation and prevents fat oxidation [3]. However, they not only increase the cost of the product but also make the environmental situation worse, as plastic waste takes too long to decompose. A solution to the problem can be to create environmentally friendly types of biodegradable packaging materials based on polysaccharide – sodium alginate [4]. This could ensure financial stability of processing enterprises, through transporting refrigerated raw materials to remote regions as well.

Our aim was to develop a progressive technology for growing lamb enriched with organic form of iodine and selenium and packed in a biodegradable sodium alginate film.

STUDY OBJECTS AND METHODS

The experiment was conducted at Saratov State Vavilov Agrarian University. We formed four groups of Edilbay lambs (10 in each) aged 4.5 months by the analog method. Feed supplements were added to the diet once a day, as directed by the guidelines, namely "Yoddar-Zn" (100g/t of feed) and "DAFS-25" (1.6 mg/kg of premixed feed). The control group received only feed in a daily amount of 250–300 g per head in addition to the main diet. The first experimental group received feed and "Yoddar-Zn". The second experimental group had feed and selenium-based "DAFS-25". The third experimental group received feed, as well as "Yoddar-Zn" and "DAFS-25" supplements.

The researchers of Volga region Research Institute of Manufacture and Processing of Meat-and-Milk Production and Vavilov Saratov State Agrarian University developed feed supplements containing essential micronutrients further enriched with protein-carbohydrate complex and Coretron mineral feed based on "Yoddar-Zn" (Specifications 10.91.10-252-10514645-2019) and "DAFS-25" (Specifications 10.91.10-253-10514645-2019). Feed supplements were added to the diet as directed by the use instructions once a day together with grain feed (barley groats) in the amount of 10% of their quantity.

Our study objects were lambs, *m. Longissimus dorsi* lamb carcasses in sodium alginate biodegradable film (experimental) and without packaging samples (control). The control and experimental samples were stored in the refrigerator chamber at –1°C and relative humidity of 85%.

The protein-carbohydrate complex included pumpkin oil cake (20%), which is the by-product of oil processing. Due to cold pressing, the pumpkin oil cake preserves the bulk of nutrients, vitamins, and trace elements, biologically active components contained in seeds, and up to 8–12% of pumpkin oil. Pumpkin oil cake is an important source of protein (up to 45% raw protein). In addition to protein, pumpkin oil cake includes sugars, phytosterol, resins, organic acids, carotenoids, thiamine, riboflavin, phosphoric and silica acid salts, potassium, calcium, iron, magnesium. A significant zinc content in pumpkin oil cake, as well as in the oil (containing glycerides linolenic, stearic, palmitic, and oleic acids) produces its favorable impact on the body's numerous functions. Following the research findings, we applied for the Patent of the Russian Federation "Feed supplement for young sheep" (application registration number: Intellectual Product 2019140759 dated 09.12.2019).

Table 1 MRM ion impact parameters and electrical field (ESI) spray ionization conditions with positive ion registration

Solute	Precursor ion, m/z	Daughter ions, m/z	Fragmentor Voltage (Frag), V	Dissociation energy (CE), V
3-iodine-L-tyrosine (MIT)	364.0	134.9	112	30
	364.0	261.9	112	13
3,5-diiodine-L-tyrosine (DIT)	489.9	387.8	116	17
	489.9	260.9	116	30

Coretron is a fine grey powder consisting of amorphous silica of biogenic origin (100%). It is used in the production of feed and premixes for farm animals and birds. Coretron prevents feed particles from caking. It has insecticidal properties, stabilizes humidity, adsorbs and excretes mycotoxins, and is a source of water-soluble silicon needed to improve calcium assimilation and provide stable functioning of animals' smooth muscles of the intestines and stomach. An important advantage of Coretron is that it eliminates product tracking, mold and mycotoxins formation, and destroys adult insect and larvae species during transportation and storage. In addition, it ensures systematic reduction of helminths in the digestive tract and saturates the body with amorphous silicon. It does not contain genetically modified ingredients. The quantity of iodine and selenium is evaluated depending on their content in the feed. Feed supplements do not violate intestinal normal flora and do not have toxic and sensitizing effects.

The main authenticity criteria for supplements based on iodized milk proteins is the presence of iodotyrosines and the degree of iodization. Obtaining organic iodine is based on enzymatic iodization of amino acid residues of cow's milk whey proteins. Until the present, the existing methods have stood on the voltammetric determination of inorganic iodine. The Federal Research Center of Food Systems named after Gorbatov (Russian Academy of Sciences) developed and certified a control method for the content of iodotyrosines in biologically active supplements (Instruction 103.5-132-2012) and State Standard 33422-2015[1]. The method allows identifying organic iodine (iodotyrosines) [10, 11]. Determination of iodotyrosines in complex food matrices containing the organic form of iodine requires the use of high-efficiency liquid chromatography technique with mass spectrometric detection (HPLC – MS/MS). The determination of organic iodine was carried out on an Agilent Technologies 6410 Triple Quadrupole system.

The conditions for recording analytical signals in the Multiple Reaction Monitoring (MRM) mode are presented in Table 1.

Confirming the presence of certain solutes requires the use of mass spectrometric identification since food and raw materials consist of organic substances that affect measurement, such as HPLC or GC. HPLC methods with classical detectors of different types (spectrophotometric, diode matrix, fluorescent, refractometric) and GC (flame ionization, electron gripping) are not selective in the determination of organic iodine and do not allow the necessary sensitivity in the study of food samples and raw materials. Voltammetry cannot be applied in the determination of iodotyrosines, as it is for analyzing inorganic iodine only. The possibility of chromatographic separation and identification of iodotyrosines in case of presence of organic impurities almost completely eliminates errors during analysis. It allows recommending HPLC – MC/MC as a reliable and highly accurate method of iodotyrosines determination in meat and meat products.

M. Longissimus dorsi from lamb carcasses was coated with sodium alginate biodegradable film (E401). Sodium alginate is used as enterosorbent: it exhibits sorption activity against heavy metals and radionuclides, as well as significantly reduces cholesterol levels in blood. In medicine, sodium alginate is used as a drug (antacid) to treat gastrointestinal diseases. Alginates have antisclerotic, immune-modulating and antimicrobial properties, improve carbohydrate metabolism, reduce lipids in the blood, and normalize thyroid function, as it contains iodine. Due to its natural origin, sodium alginate has a high safety level for humans, certified for the production of baby food.

Calcium chloride is a dietary supplement (E509) that refers to emulsifiers and is a drug that complements the lack of calcium in the body. It is generally used together with other hydrocolloids: carrageenans, pectin, and most often with sodium alginate, which needs its ions to form biodegradable food coatings for meat raw materials.

The mechanism for alginate gels formation involves the joint binding of calcium ions between single line polygaluronate sequences. The chains of macromolecules bound in such a configuration have pores or cavities corresponding to the size of the Ca^{+2} ion radius. Gelling is intense when filling pores with calcium ions.

To produce biodegradable film, we prepared a homogeneous 2% solution that was constantly mixed at 150–200 rpm. Alginate film formed as a result of spaying 0.05% calcium chloride (pH below 3.6). Calcium chloride, interacting with sodium alginate, forms a thin stable, thixotropic, transparent protective coating. To accelerate the formation of the coating outer layer, it was fixed by the flow of air in the refrigerating chamber.

The mineral composition of lamb muscle tissue was assessed for the presence of micronutrients (Se, Zn, I). Macro- and micronutrients were determined by atomic

[1] State Standard 33422-2015. Meat and meat products. Determination of iodotyrosines using high performance liquid chromatography with mass spectrometry detection. Moscow: Standartinform; 2016. 10 p.

Table 2 Content of toxic elements in lamb grown on enriched diets

Element	Content, μg/g			
	Control group	Group I (iodine)	Group II (selenium)	Group III (iodine, selenium)
Arsenic (As)	0.002 ± 0.0004	0.003 ± 0.0007	0.003 ± 0.0005	0.002 ± 0.0005
Cadmium (Cd)	< 0.00048	0.001 ± 0.0003	0.0005 ± 0.00015	< 0.00048
Lead (Pb)	0.008 ± 0.0016	0.007 ± 0.0013	0.01 ± 0.002	0.01 ± 0.002

absorption spectroscopy (State Standard 53182-2008[II], State Standard 31660-2012[III], State Standard 30178-96[IV], State Standard 33422-2015[V]).

Immuno-physiological parameters of blood were analyzed by standard methods: the number of red and white blood cells was counted in Goryaev's chamber, haemoglobin was determined by Sahli's method, total serum protein was refractometrically measured by McCord, phagocytic number, phagocytic activity, and phagocytic intensity Mancini method.

Toxic elements – lead, cadmium and arsenic – were determined according to Methodological Guidelines 4.1.986 "Methods of measuring the mass fraction of lead and cadmium in food and food raw materials by electrothermal atomic absorption spectrometry"[VI] established by the Scientific Council for Analytical Methods 450×. Statistical processing of the results on the dynamics of changes in hematological and biochemical parameters of blood serum was carried out according to standard procedures, using the Microsoft Excel application 2010 (Microsoft Corp. USA) and the StatPlus 2009 Professional 5.8.4 for Windows statistical data analysis package (StatSoft Inc., USA), with the Student *t* criterion applied to assess the validity of differences between experimental and control samples. Based on the arithmetic mean and standard deviation, we determined the standard error of the arithmetic mean and the boundary of its confidence interval, taking into account the coefficient t (n, p) at a significance level of 95% (P = 0.05) and number of measurements. The significance of differences between the average values in the experimental and control tests was assessed by the *P*-value in the variant of a two-sample unpaired *t*-test with unequal variances. The differences were considered significant at $P \geq 0.05$. In addition, we observed the inequality t, $t(n, p)$ at $n = (df + 1)$ (where df is the number of degrees of freedom), $P = 0.05$, where

$$t = \frac{|x_1 - x_2|}{(s_1^2 + s_2^2)^{1/2}}$$

where x_1 and x_2 are arithmetic mean values, s_1 and s_2 are their standard errors for two experimental data samples [12].

RESULTS AND DISCUSSION

High productivity of small cattle is impossible without rational and full-value feeding based on the knowledge of physiological state, level of productivity, intended use and need in energy, nutrients, minerals, vitamins and other biologically active substances. Highly important is to balance diets in minerals and micronutrients, especially in the regions where their content is low.

To optimize the chemical composition of lamb *in vivo*, we studied the efficiency of feed enriched with organic forms of iodine, selenium, and zinc ("Yoddar-Zn" and "DAFS-25"), when rearing small cattle.

At the initial stage, we performed a sanitary examination of lamb meat from the animals grown on enriched diets. The content of toxic elements in the lamb under study is presented in Table 2.

The findings showed that the content of toxic elements in the lamb from experimental groups complied with the requirements of the Technical Regulations of the Customs Union "On meat and meat products safety" (TR CU 034/2013[VII]).

The study of "DAFS-25" and "Yoddar-Zn" effects, as well as their combined effect on the lambs' resistance, showed that the hematological indicators of the animals were within physiological norms. At the same time, blood morphological composition and biochemical parameters showed intergroup differences (Table 3).

We detected that hemoglobin was higher ($P > 0.99$) in the lambs of the experimental groups. The concentration of total protein during the same period was slightly lower, which might be driven by more intensive protein exchange processes and better growth energy. No reliable differences were established in the groups in terms of blood cells (Table 3).

[II] State Standard 53182-2008. Foodstuffs. Determination of trace elements. Determination of total arsenic and selenium by hydride generation atomic absorption spectrometry (HGAAS) method after pressure digestion. Moscow: Standartinform; 2010. 16 p.

[III] State Standard 31660-2012. Foods. Anodic stripping voltammetric method of iodine mass concentration determination. Moscow: Standartinform; 2012. 15 p.

[IV] State Standard 30178-96. Raw material and food-stuffs. Atomic absorption method for determination of toxic elements. Moscow: Standartinform; 2010. 8 p.

[V] State Standard 33422-2015. Meat and meat products. Determination of iodotyrosines using high performance liquid chromatography with mass spectrometry detection. Moscow: Standartinform; 2016. 10 p.

[VI] MUK 4.1.986-00 Metodika vypolneniya izmereniy massovoy doli svintsa i kadmiya v pishchevykh produktakh i prodovol'stvennom syr'e metodom ehlektrotermicheskoy atomno-absorbtsionnoy spektrometrii [MG 4.1.986. Methods of measuring the mass fraction of lead and cadmium in food and food raw materials by electrothermal atomic absorption spectrometry]. Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of Russia; 2000. 32 p.

[VII] TR TS 034/2013. Tekhnicheskiy reglament Tamozhennogo soyuza "O bezopasnosti myasa i myasnoy produktsii" [TR CU 034/2013. Technical Regulations of the Customs Union "On meat and meat products safety"]. 2013. 108 p.

Table 3 Immuno-physiological blood indicators of lambs on different diets

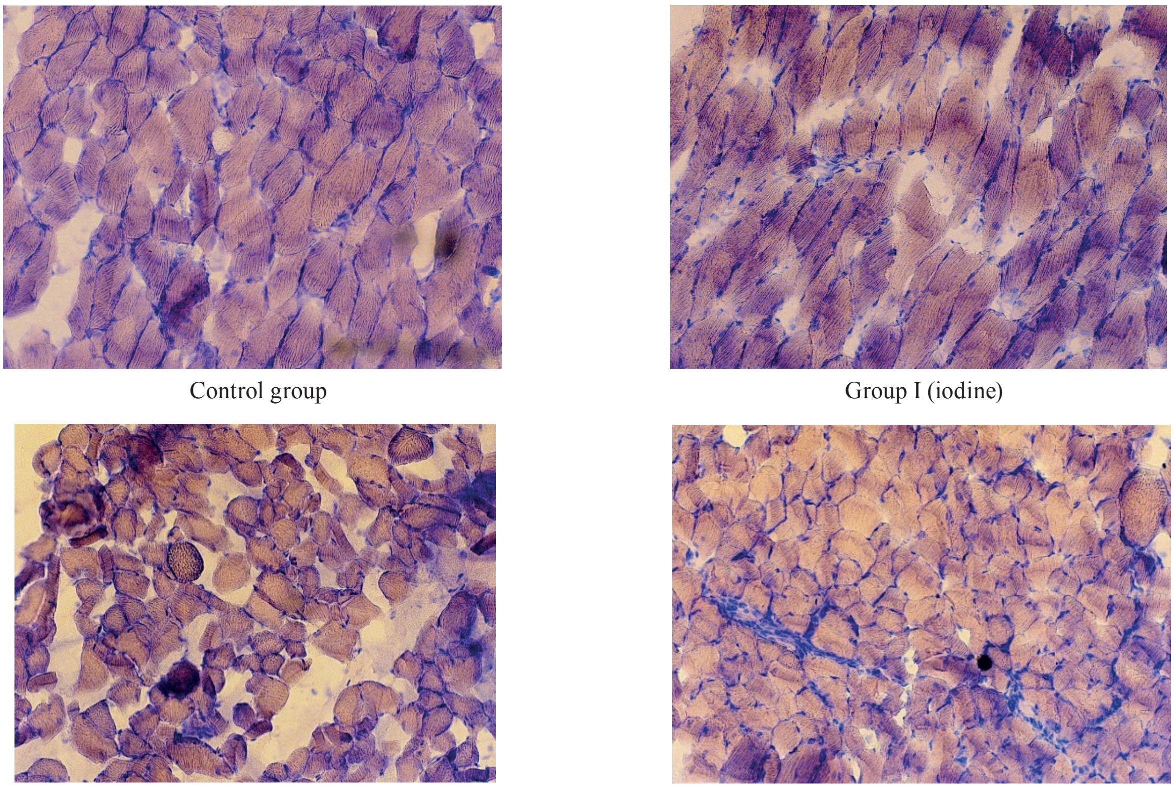
Group / Indicator	Lambs 4.5 months old (n = 25)				Lambs 7.5 months old (n = 25)			
	Control group	Group I (iodine)	Group II (selenium)	Group III (iodine, selenium)	Control group	Group I (iodine)	Group II (selenium)	Group III (iodine, selenium)
Erythrocytes, mln/μL	14.30 ± 0.03	14.60 ± 0.02	13.90 ± 0.03	14.00 ± 0.01	10.01 ± 0.01	10.40 ± 0.02	10.07 ± 0.02	10.70 ± 0.02
Leukocytes, thousand/μL	4.00 ± 0.02	3.9 0 ± 0.01	4.10 ± 0.02	4.20 ± 0.02	8.21 ± 0.01	8.25 ± 0.01	8.26 ± 0.01	8.28 ± 0.02
Hemoglobin, g/L	130.00 ± 0.01	139.00 ± 0.01	134.00 ± 0.01	129.00 ± 0.01	128.00 ± 0.04	131.20 ± 0.07	132.70 ± 0.03	133.20 ± 0.09
Total protein, g	85.30 ± 0.09	84.90 ± 0.03	85.30 ± 0.05	85.30 ± 0.04	69.20 ± 0.02	64.00 ± 0.07	62.10 ± 0.04	61.00 ± 0.01
Phagocytic number, unit	1.33 ± 0.02	1.38 ± 0.02	1.30 ± 0.02	1.32 ± 0.02	1.38 ± 0.02	1.45 ± 0.01	1.51 ± 0.02	1.58 ± 0.02
Phagocytic activity,%	56.80 ± 0.03	53.40 ± 0.03	52.90 ± 0.03	56.10 ± 0.03	55.70 ± 0.01	57.70 ± 0.02	59.20 ± 0.03	59.70 ± 0.02
Phagocytic intensity, unit	2.33 ± 0.03	2.17 ± 0.03	2.26 ± 0.03	2.20 ± 0.03	2.34 ± 0.01	2.46 ± 0.05	2.55 ± 0.02	2.70 ± 0.02

The lambs of the experimental groups had a higher phagocytic number compared to the control, whose diet included only feed: 5.1% ($P > 0.95$), 9.4% ($P > 0.95$), and 14.5% ($P > 0.99$) in groups I, II, III, respectively. In addition, the meat of group II and group III had increased phagocytic activity and phagocytic intensity. This indicated their higher resistance to adverse environmental factors compared to the control group.

Thus, selenium and iodine feed supplements in the diet of lambs in the early post-embryonic period stimulate their development and increase resistance.

Meat quality is largely determined by the histological structure of animal muscle tissue and depends on the size of muscle fibers, as well as condition and structure of connective and adipose tissues. To assess the meat quality, we studied the changes in the histological structure of *m. Longissimus dorsi* carcasses of the four lamb groups depending on their diet.

The muscle tissues of all the studied lambs grown on various diets had morphological features characteristic of the beginning of rigidity. We detected no significant differences in muscle tissues in the course of autolysis.



Control group

Group I (iodine)

Group II (selenium)

Group III (iodine and selenium)

Figure 1 Microstructure of lamb tissue (*m. Logissimus dorsi*) grown on different diets

Table 4 Average daily growth of living lamb mass (M ± m)

Age, months	Mass growth, g (n = 10)			
	Control group	Group I (iodine)	Group II (selenium)	Group III (iodine and selenium)
4.5–7.5	111.67 ± 0.12	115.50 ± 0.22	119.17 ± 0.32	128.33 ± 0.22

Table 5 Chemical composition of lamb meat obtained from animals on different fattening diets

Group	Moisture, %	Dry substance, %	Protein, %	Fat, %	Ash, %	Energy value/kg, kJ
Control	71.12 ± 0.22	28.88	18.23 ± 0.12	9.60 ± 0.10	1.05 ± 0.02	8167.60
Group I (iodine)	71,01 ± 0,31	28.98	18.13 ± 0.11	9.84 ± 0.13	1.01 ± 0.01	8239.19
Group II (selenium)	70.85 ± 0.25	29.15	18.06 ± 0.14	10.11 ± 0.17	0.98 ± 0.01	8329.87
Group III (iodine and selenium)	69.96 ± 0.23	30.04	17.94 ± 0.12	11.17 ± 0.14	0.95 ± 0.01	8718.02

The micro-structural analysis revealed that the use of "DAFS-25" and "Yoddar-Zn" in raising young sheep did not cause any negative changes in muscular fibers and surrounding connective tissue, endomysium and perimysium. This finding indicated that they can be used for fattening animals in industrial production.

The average daily growth of live mass among lambs aged 4.5–7.5 months showed that the animals in the experimental groups had higher weight than those in the control group. In particular, the differences were 3.83 g (3.43%) in group I ($P \geq 0.999$), 7.50 g (6.72%) in group II ($P \geq 0.999$), and 16.66 g (14.92%) in group III ($P \geq 0.999$) (Table 4).

In our opinion, the mass growth was due to the diet enrichment with organic selenium and iodine.

The chemical composition of lamb is shown in Table 5.

The meat of the experimental groups had a better composition. With equal protein and ash content, it contained less water and had higher caloricity (Table 4).

Mineral substances are a structural material for tissues and organs. They are a part of organic substances participating in respiration, hemotogenesis, digestion, absorption, synthesis, flow, and release of metabolism products from the body. They are interrelated with the activity of many biologically active substances and generally affect metabolism and numerous physiological functions of the organism. Iodine, selenium, and zinc are of high importance in the metabolism of the organism.

The content of zinc, iodine, and selenium in lamb was directly dependent on their content in the diets, with a higher content recorded in lamb of group III (Fig. 2).

Soft enzymatic hydrolysis, chromatography and mass spectrometry were used to identify and assess the site of iodine incorporation into proteins. We found no changes in hydrolysis of lamb proteins involving proteolytic enzymes, unlike acid hydrolysis. There were no changes in hydrolysis products. Figures 3, 4 show the chromatograms of the lamb samples with iodotyrosines.

Iodotyrosine determination can be performed using diode array or spectrophotometric detectors. However, when the target iodotyrosines are extracted from compounds such as food products, the finished samples may contain foreign organic impurities. In some cases, while using diode array or spectrophotometric detectors, mistakes may occur due to the presence of cross-signal substances. Determining separate iodotyrosines may also be difficult due to incomplete separation of chromatographic peaks.

We should take into account that a column is not capable of separating iodotyrosines in the presence of organic compounds as well as it works without foreign impurities. Liquid chromatography with mass spectrometric detection (HPLC – MC/MC) is a more reliable method to determine iodotyrosines in food products than HPLC with diode array or spectrophotometric detectors. Its advantage is high sensitivity toward the components under examination.

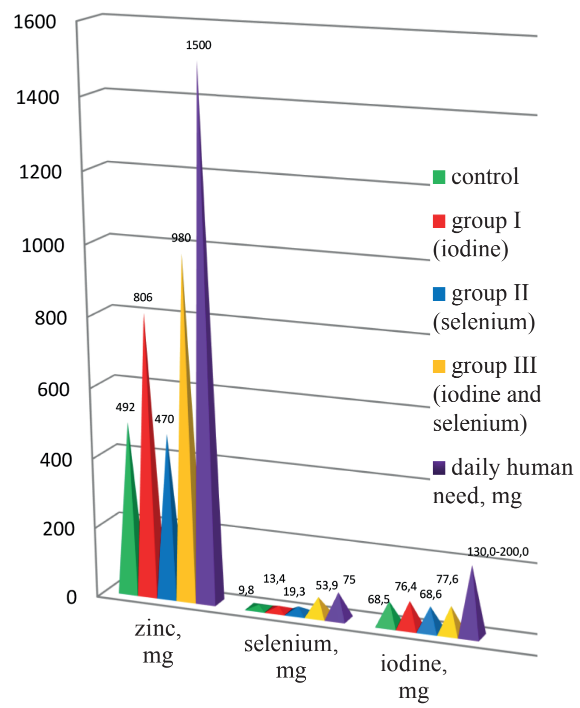


Figure 2 Zinc, selenium, and iodine contents in 100 g of lamb depending on the diet

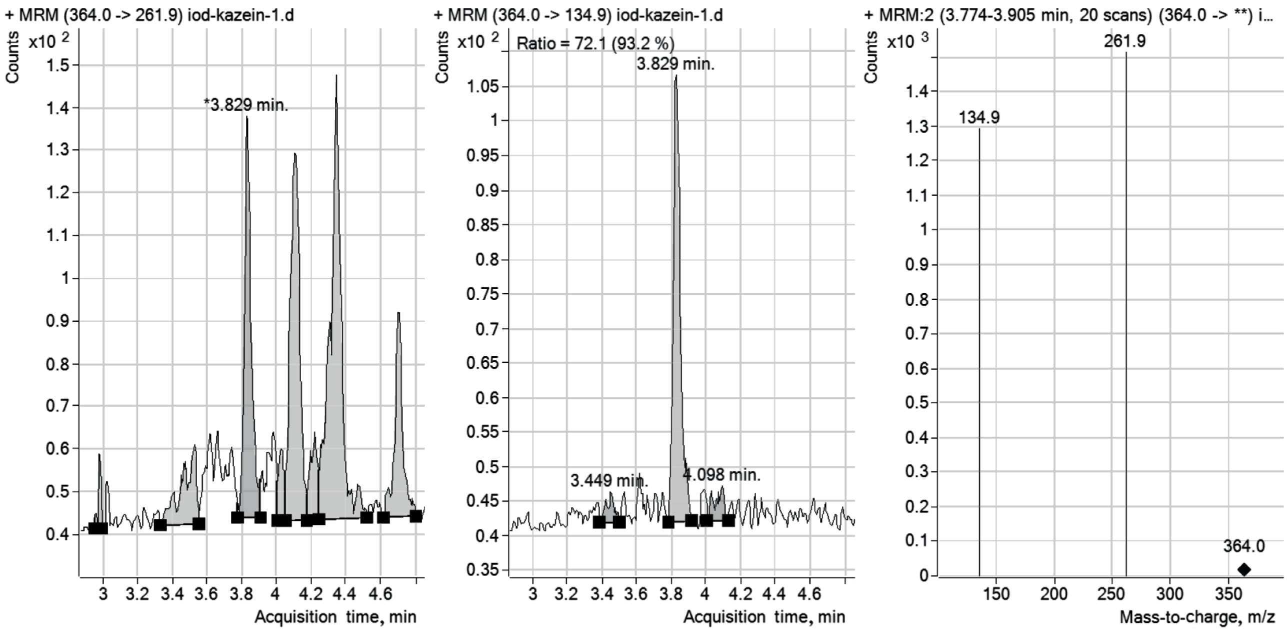


Figure 3 Chromatogram of a lamb sample with iodthyrosines (total ion current and MRM transitions of monoiodotyrosine)

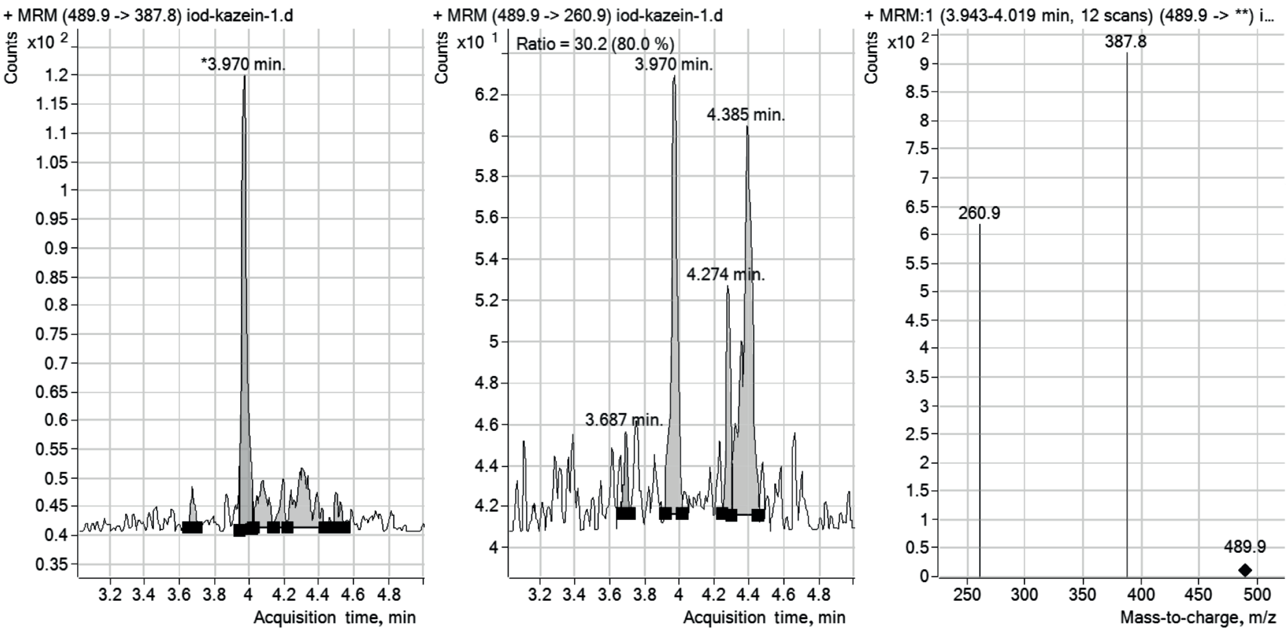


Figure 4 Chromatogram of a lamb sample with iodotyrosines (total ion current and MRM transitions of diiodotyrosine)

The selective ion detection mode eliminates false-positive results in case of cross-signal substances present in samples. The monitoring mode of daughter ions allows determining of the compounds when their molecular masses match.

In the course of analysis, we tested the method of ionization by spraying in the electric field (ESI) with pre-column derivatization of iodotyrosines by a butanol:acetylchloride mixture (4:1). As shown in Figs. 3, 4, the chromatograms of the lamb samples with an iodotyrosine content are presented in the mode of monitoring daughter ions on the three-quadrupole mass detector Agilent 6410. The confirming ions for these substances were different; solutes were chromatographically separated with a fine-grained column with C18 phase (Agilent Eclipse XDB C18, 4.6×50 mm, 1.8 μm).

Thus, we identified organic iodine and determined its quantity in the form of iodotyrosines. It was due to its covalently bound form that organic iodine was able to exhibit many biological properties, including through iodine-containing hormones, thyroxine and

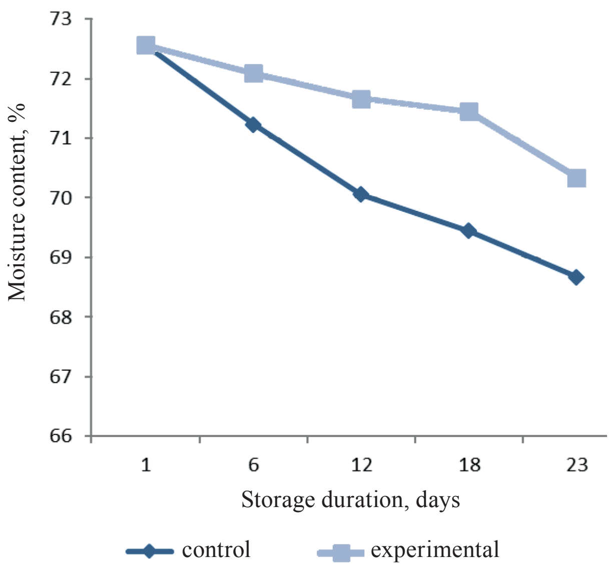


Figure 5 Loss of moisture in experimental and control lamb groups during storage

triiodothyronine, involved in the regulation of all metabolic processes in the human body.

We found that the use of mineral supplements in the diets of small cattle is promising and relevant. It allows obtaining lamb enriched with organic trace elements.

The duration of lamb storage under refrigerator conditions is limited by a number of factors, including temperature, humidity, carcass contamination, post-mortal biochemical processes, as well as the presence of eco-toxicants and residues of medicines in meat [13]. To eliminate the negative impact of refrigeration on meat quality and extend the shelf life, we covered the cuts with biodegradable film based on sodium alginate.

Sodium alginate film is homogeneous in structure, flexible, and transparent. It has structural resistance and barrier effect against air oxygen and microorganisms. Biodegradable package does not lose its structure-forming properties after refrigeration; there is no need to remove it before use. An important advantage of bio-film is sensory acceptability and low cost [14].

Loss of meat mass due to moisture evaporation during cooling and freezing is not only a quantitative characteristic. The surface of meat after draining becomes porous, with temperature burns resulting in the deterioration of commercial appearance. Meat easily absorbs foreign odors and oxidation processes accelerate. Moisture losses during lamb cooling in biodegradable film are presented in Fig. 5.

Moisture loss during storage of the control samples (lamb without coating) was 3.71% higher than in the experimental group (lamb in biodegradable film). This confirmed that biodegradable film provides dense adhesion to the surface of raw materials, which prevents moisture exchange and, therefore, minimizes moisture loss [15].

CONCLUSION

The use of selenium and iodine supplements, namely "Yoddar-Zn" and "DAFS-25", in small cattle diets stimulated their development, increased their resistance and productivity, and enriched lamb meat with organic zinc, selenium, and iodine. This has a big medical and social importance for preventing micronutrient deficiency in the population.

We found that the hematological indicators of animals grown on enriched diets were within physiological norms. The content of hemoglobin was higher in the experimental groups ($P > 0.99$). The concentration of total protein during the same period was slightly lower, which might be driven by more intensive protein exchange processes and better growth energy. No reliable differences were established in the content of red blood cells in the groups (Table 3).

We detected a higher phagocytic number in the experimental groups. Also, the meat of group II and group III exceeded the control group for phagocytic activity and intensity. This indicated their higher resistance to adverse environmental factors compared to the control group.

We determined organic iodine and its quantity in the form of iodotyrosines. It was due to its covalently bound form that organic iodine was able to exhibit many biological properties, including through iodine-containing hormones – thyroxine and triiodothyronine – involved in the regulation of all metabolic processes in the human body. Monitoring trace elements showed that zinc, iodine, and selenium contents in lamb meat directly depended on their amounts in the lambs' diet. We detected their higher concentration in lamb meat of the 3[d] experimental group (iodine and selenium diet).

The microstructural analysis revealed that the use of "DAFS-25" and "Yoddar-Zn" in raising young lambs did not cause any negative changes in muscular fibers, endomysium and perimysium. Thus, they can be used for fattening animals in industrial production.

CONTRIBUTION

Authors are equally related to the writing of the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest

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